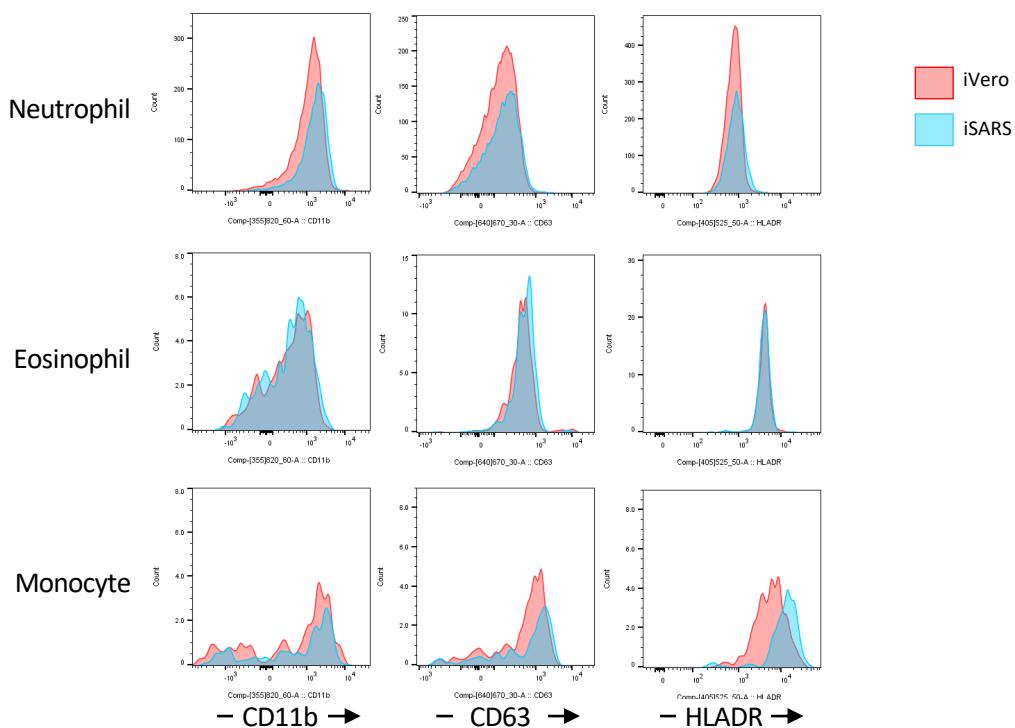


## **Off-target effects of BCG vaccination on immune responses to SARS-CoV-2: implications for protection against severe COVID-19**

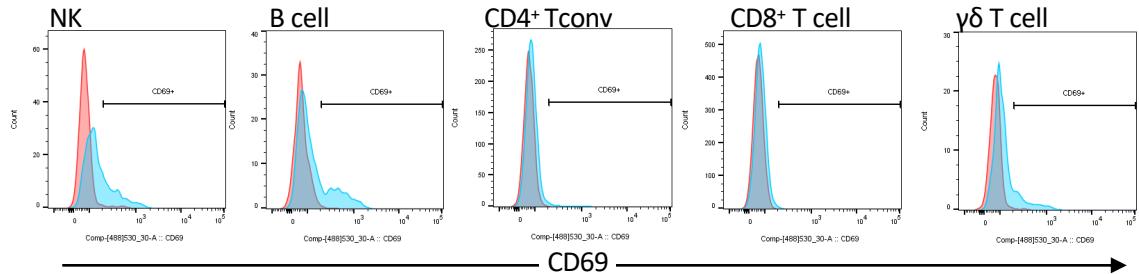
Nicole L Messina, Susie Germano, Rebecca McElroy, Rajeev Rudraraju, Rhian Bonnici, Laure F Pittet, Melanie R Neeland, Suellen Nicholson, Kanta Subbarao, Nigel Curtis, the BRACE trial Consortium Group

### **Supporting information**

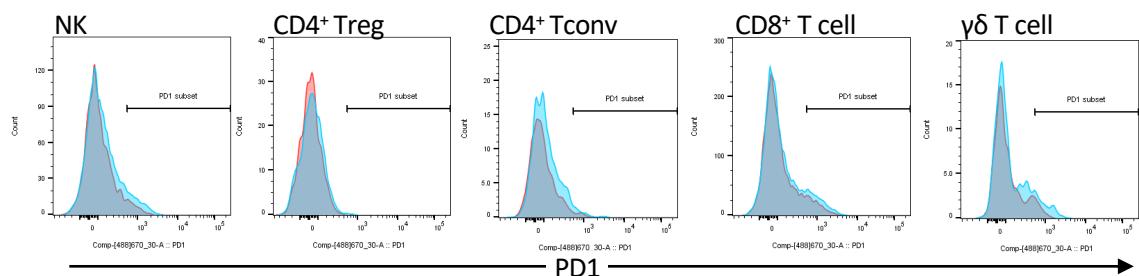
(a)



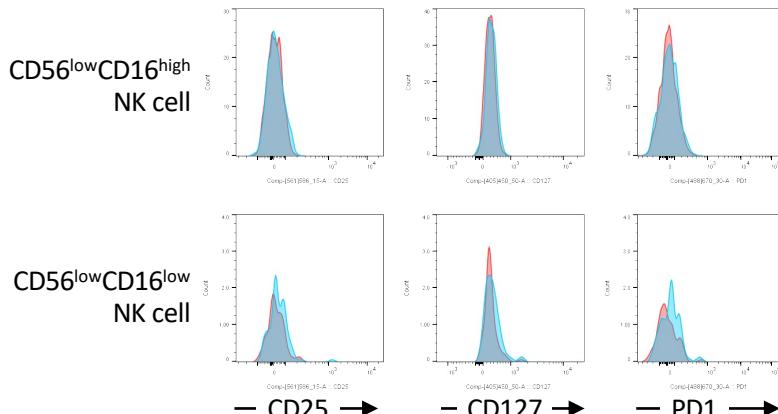
(b)



(c)



(d)

**Supplementary figure 1:** Representative histograms of iSARS stimulation effect.

Overlay histograms of one representative sample showing cell surface marker expression on immune cell subpopulations in iVero (red) and iSARS (blue) stimulated whole blood samples.

**Supplementary table 1: Difference (3-months - baseline) in stimulation effect on cytokine responses**

| Cytokine        | BCG group |         |         |              | Placebo group |         |         |              |
|-----------------|-----------|---------|---------|--------------|---------------|---------|---------|--------------|
|                 | N         | median  | IQR     | P-value      | N             | median  | IQR     | P-value      |
| CTACK           | 28        | 6.6     | 96.7    | 0.767        | 22            | 33.1    | 133.1   | 0.168        |
| Eotaxin         | 28        | -1.8    | 14.7    | 0.466        | 22            | 0.1     | 9.7     | 0.733        |
| FGF basic       | 28        | -25.9   | 33.5    | <b>0.011</b> | 22            | -9.3    | 59.5    | 0.833        |
| G-CSF           | 28        | -485.1  | 1490.6  | <b>0.005</b> | 22            | 199.0   | 1881.9  | 0.485        |
| GM-CSF          | 28        | -1.8    | 5.7     | <b>0.010</b> | 22            | 2.4     | 8.2     | 0.101        |
| GRO- $\alpha$   | 28        | -135.1  | 492.4   | 0.080        | 22            | 119.9   | 353.6   | 0.277        |
| HGF             | 28        | -99.0   | 142.7   | <b>0.017</b> | 22            | -25.1   | 217.6   | 0.833        |
| IFN- $\alpha$ 2 | 28        | -169.2  | 432.0   | <b>0.002</b> | 22            | -133.5  | 431.9   | <b>0.004</b> |
| IFN $\gamma$    | 28        | -44.2   | 85.5    | <b>0.005</b> | 22            | -48.8   | 66.4    | <b>0.009</b> |
| IL-1 $\alpha$   | 28        | -7.3    | 31.9    | 0.172        | 22            | 7.6     | 38.0    | 0.249        |
| IL-1 $\beta$    | 28        | -2.7    | 12.0    | 0.080        | 22            | 0.7     | 13.3    | 0.808        |
| IL-1ra          | 28        | -3558.3 | 5051.0  | <b>0.000</b> | 22            | -2861.0 | 5735.6  | <b>0.003</b> |
| IL-2            | 28        | 1.1     | 14.4    | 0.847        | 22            | 0.0     | 15.2    | 0.346        |
| IL-2R $\alpha$  | 28        | -2.8    | 83.5    | 0.387        | 22            | 16.0    | 77.5    | 0.338        |
| IL-3            | 28        | 0.0     | 0.0     | 0.979        | 22            | 0.0     | 0.0     | 0.974        |
| IL-4            | 28        | -2.3    | 4.7     | <b>0.017</b> | 22            | -0.7    | 4.5     | 0.527        |
| IL-5            | 28        | 0.0     | 45.7    | 0.419        | 22            | 12.2    | 120.4   | 0.170        |
| IL-6            | 28        | -39.1   | 121.0   | <b>0.036</b> | 22            | 2.8     | 153.2   | 0.961        |
| IL-7            | 28        | 0.0     | 5.4     | 0.876        | 22            | 0.0     | 21.4    | 0.099        |
| IL-8            | 28        | -76.5   | 238.8   | <b>0.036</b> | 21            | -118.3  | 354.7   | <b>0.023</b> |
| IL-9            | 28        | -50.8   | 198.3   | 0.076        | 22            | -23.0   | 211.9   | 0.910        |
| IL-10           | 28        | -7.2    | 17.6    | <b>0.021</b> | 22            | -5.3    | 18.0    | 0.140        |
| IL-12p40        | 28        | -23.5   | 110.8   | 0.158        | 22            | 23.5    | 101.2   | 0.205        |
| IL-12p70        | 28        | 0.0     | 3.2     | 0.567        | 22            | 0.0     | 6.4     | 0.375        |
| IL-13           | 28        | 0.0     | 2.6     | 0.465        | 22            | 0.4     | 2.1     | 0.167        |
| IL-15           | 28        | -3.3    | 468.5   | 0.973        | 22            | 60.4    | 615.9   | 0.178        |
| IL-16           | 28        | -10.8   | 49.3    | 0.133        | 22            | 4.9     | 54.5    | 0.485        |
| IL-17           | 28        | 0.0     | 22.3    | 0.954        | 22            | -1.5    | 26.4    | 0.548        |
| IL-18           | 28        | 7.1     | 19.5    | 0.151        | 22            | 4.6     | 21.3    | 0.123        |
| IP-10           | 26        | -6236.7 | 26968.5 | 0.066        | 21            | -8480.9 | 28894.0 | <b>0.039</b> |
| LIF             | 28        | -46.5   | 182.2   | <b>0.048</b> | 22            | 16.1    | 273.7   | 0.661        |
| MCP-1           | 26        | -569.1  | 4282.3  | 0.732        | 18            | 637.8   | 4187.8  | 0.679        |
| MCP-3           | 28        | 11.7    | 436.2   | 0.495        | 22            | 41.6    | 410.8   | 0.506        |
| M-CSF           | 28        | -2.0    | 18.0    | 0.466        | 22            | 0.9     | 15.6    | 0.638        |
| MIF             | 28        | -76.7   | 786.6   | 0.802        | 22            | -56.0   | 543.2   | 0.910        |
| MIG             | 28        | -90.8   | 163.1   | <b>0.024</b> | 22            | -20.2   | 153.9   | <b>0.050</b> |
| MIP-1 $\alpha$  | 27        | -6.3    | 61.1    | 0.118        | 20            | 3.3     | 52.6    | 0.502        |
| MIP-1 $\beta$   | 27        | -470.4  | 1211.6  | <b>0.003</b> | 22            | 59.7    | 910.6   | 0.426        |
| $\beta$ -NGF    | 28        | -5.0    | 24.0    | 0.374        | 22            | 2.8     | 24.7    | 0.408        |
| PDGF-BB         | 28        | -60.0   | 262.1   | 0.194        | 22            | 40.7    | 253.0   | 0.211        |
| RANTES          | 28        | -362.4  | 1152.7  | <b>0.012</b> | 22            | 107.6   | 724.3   | 0.465        |
| SCF             | 28        | -12.2   | 37.5    | 0.127        | 22            | 4.2     | 45.5    | 0.306        |
| SCGF- $\beta$   | 28        | -2100.2 | 36701.4 | 0.891        | 22            | 10848.1 | 22242.7 | <b>0.010</b> |
| SDF-1 $\alpha$  | 28        | -6.1    | 261.0   | 0.927        | 22            | -26.2   | 167.1   | 0.638        |
| TNF- $\alpha$   | 28        | -45.7   | 119.4   | <b>0.023</b> | 22            | 20.2    | 138.4   | 0.322        |
| TNF- $\beta$    | 28        | -62.9   | 246.6   | <b>0.021</b> | 22            | -30.1   | 249.5   | 0.758        |
| TRAIL           | 28        | -7.2    | 83.5    | 0.116        | 22            | 7.3     | 105.9   | 0.372        |
| VEGF            | 28        | -62.9   | 531.9   | 0.219        | 22            | -39.0   | 1086.8  | 0.372        |

SE = stimulation effect (iSARS-iVERO)

**Supplementary table 2: Difference (3-months - baseline) in stimulation effect on immune cell populations**

| Parameter                | Frequency of           | BCG group |        |      |         | Placebo group |        |      |         |
|--------------------------|------------------------|-----------|--------|------|---------|---------------|--------|------|---------|
|                          |                        | N         | median | IQR  | P-value | N             | median | IQR  | P-value |
| B cells                  | CD45+                  | 9         | -0.5   | 1.3  | 0.314   | 8             | 0.1    | 0.5  | 0.263   |
| CD4+ (total)             | CD45+                  | 9         | -3.9   | 6.4  | 0.314   | 8             | -1.1   | 3.7  | 0.401   |
| CD8+ T cells             | CD45+                  | 9         | -1.0   | 1.2  | 0.086   | 8             | -0.7   | 5.2  | 0.889   |
| Dendritic cells          | CD45+                  | 9         | 0.0    | 0.1  | 0.515   | 8             | 0.0    | 0.1  | 0.484   |
| Eosinophils              | CD45+                  | 9         | -0.7   | 1.7  | 0.859   | 8             | 0.1    | 1.2  | 0.674   |
| Granulocytes             | CD45+                  | 9         | 5.0    | 12.7 | 0.214   | 8             | -1.0   | 13.2 | 0.674   |
| Monocytes                | CD45+                  | 9         | 0.2    | 0.7  | 0.038   | 8             | 0.3    | 0.5  | 0.017   |
| Mononuclear cells        | CD45+                  | 9         | -7.1   | 7.0  | 0.110   | 8             | -1.4   | 12.6 | 0.889   |
| Neutrophils              | CD45+                  | 9         | 5.3    | 9.8  | 0.173   | 8             | 0.2    | 14.5 | 0.779   |
| NK cells cells           | CD45+                  | 9         | -0.6   | 0.8  | 0.260   | 8             | -0.6   | 3.5  | 0.889   |
| Non conventional T cells | CD45+                  | 9         | -1.1   | 1.8  | 0.038   | 8             | -1.5   | 1.9  | 0.093   |
| T cells                  | CD45+                  | 9         | -4.7   | 6.4  | 0.110   | 8             | -4.4   | 5.9  | 0.161   |
| B cells                  | Mononuclear cells      | 9         | 0.4    | 4.5  | 0.678   | 8             | 0.1    | 0.9  | 0.779   |
| CD56-CD16+               | Mononuclear cells      | 9         | 0.1    | 1.3  | 0.374   | 8             | 0.4    | 1.2  | 0.401   |
| Dendritic cells          | Mononuclear cells      | 9         | 0.0    | 0.1  | 0.260   | 8             | 0.0    | 0.2  | 0.889   |
| Monocytes                | Mononuclear cells      | 9         | 0.7    | 2.8  | 0.374   | 8             | -0.1   | 1.5  | 0.674   |
| NK cells                 | Mononuclear cells      | 9         | -0.5   | 1.6  | 0.260   | 8             | -0.2   | 0.9  | 0.362   |
| T cells                  | Mononuclear cells      | 9         | -10.7  | 9.2  | 0.314   | 8             | -6.5   | 11.4 | 0.012   |
| Classical                | Monocytes              | 9         | -8.0   | 8.6  | 0.011   | 8             | -4.9   | 18.6 | 0.208   |
| Intermediate             | Monocytes              | 9         | 28.6   | 15.7 | 0.008   | 8             | 21.8   | 25.1 | 0.036   |
| Non Classical            | Monocytes              | 9         | -17.8  | 21.4 | 0.028   | 8             | -10.6  | 22.4 | 0.069   |
| CD4+ Tconv               | T cells                | 9         | -3.1   | 6.0  | 0.515   | 8             | -1.2   | 6.0  | 0.208   |
| CD8+ T cells             | T cells                | 9         | 1.0    | 12.9 | 0.767   | 8             | 1.8    | 6.7  | 0.575   |
| Non conventional T cells | T cells                | 9         | 0.3    | 7.7  | 0.515   | 8             | 1.9    | 5.1  | 0.484   |
| Tregs                    | T cells                | 9         | -0.1   | 0.7  | 0.859   | 8             | -0.6   | 0.6  | 0.036   |
| $\gamma\delta$ T cells   | T cells                | 9         | 0.1    | 1.0  | 0.767   | 8             | 0.1    | 0.7  | 0.401   |
| CD69+                    | B cells                | 9         | 3.2    | 13.2 | 0.767   | 8             | -1.4   | 11.8 | 0.889   |
| CD69+                    | CD4+ Tconv             | 9         | 1.1    | 1.9  | 0.441   | 8             | -0.6   | 1.2  | 0.401   |
| CD69+                    | CD56-CD16+             | 5         | -8.7   | 5.1  | 0.043   | 5             | -7.8   | 11.8 | 0.345   |
| CD69+                    | CD8+ T cells           | 9         | 2.9    | 4.7  | 0.066   | 8             | 1.1    | 6.9  | 0.327   |
| CD69+                    | NK cells               | 9         | -6.2   | 5.7  | 0.086   | 8             | -7.4   | 22.7 | 0.161   |
| CD69+                    | $\gamma\delta$ T cells | 9         | -1.0   | 14.3 | 0.678   | 8             | -9.5   | 19.9 | 0.161   |
| PD1+                     | CD4+ Tconv             | 9         | 0.5    | 2.0  | 0.008   | 8             | -0.7   | 3.0  | 0.263   |
| PD1+                     | CD8+ T cells           | 9         | 0.7    | 4.9  | 0.515   | 8             | -0.4   | 1.4  | 0.263   |
| PD1+                     | NK cells               | 9         | -0.4   | 1.1  | 0.139   | 8             | 0.3    | 1.2  | 0.889   |
| PD1+                     | Tregs                  | 9         | 5.4    | 12.5 | 0.374   | 8             | 1.3    | 9.3  | 0.674   |
| PD1+                     | $\gamma\delta$ T cells | 9         | -0.3   | 10.5 | 0.594   | 8             | -2.7   | 13.5 | 0.401   |
| Naïve                    | CD4+ Tconv             | 9         | -3.1   | 4.0  | 0.011   | 8             | -0.6   | 11.1 | 0.889   |
| Effector memory          | CD4+ Tconv             | 9         | 3.6    | 1.2  | 0.028   | 8             | 3.3    | 8.2  | 0.123   |
| Central memory           | CD4+ Tconv             | 9         | -1.5   | 3.4  | 0.594   | 8             | -2.3   | 5.6  | 0.069   |
| TEMRA                    | CD4+ Tconv             | 9         | 0.6    | 2.8  | 0.374   | 8             | -1.0   | 4.0  | 0.575   |
| Naïve                    | CD8+ T cells           | 9         | -3.1   | 5.2  | 0.038   | 8             | 1.4    | 6.9  | 0.779   |
| Effector memory          | CD8+ T cells           | 9         | 4.9    | 3.0  | 0.008   | 8             | -4.2   | 11.4 | 0.575   |
| Central memory           | CD8+ T cells           | 9         | -1.0   | 3.4  | 0.260   | 8             | 0.1    | 4.3  | 0.779   |
| TEMRA                    | CD8+ T cells           | 9         | 0.1    | 4.2  | 0.767   | 8             | 2.0    | 4.3  | 0.327   |
| Naïve                    | Tregs                  | 9         | -7.6   | 13.1 | 0.038   | 8             | 8.4    | 22.4 | 0.674   |
| Activated                | Tregs                  | 9         | 7.0    | 20.1 | 0.173   | 8             | -10.0  | 25.3 | 0.779   |
| CD56hiCD16-              | NK cells               | 9         | -1.1   | 5.6  | 0.953   | 8             | 2.7    | 5.5  | 0.779   |
| CD56hiCD16hi             | NK cells               | 9         | 0.9    | 0.9  | 0.173   | 8             | 0.3    | 3.1  | 0.674   |
| CD56loCD16-              | NK cells               | 9         | 0.5    | 2.2  | 0.767   | 8             | -0.6   | 5.0  | 0.674   |
| CD56loCD16hi             | NK cells               | 9         | 1.2    | 11.8 | 0.953   | 8             | 0.5    | 7.5  | 0.624   |
| CD56loCD16lo             | NK cells               | 9         | -0.5   | 12.1 | 0.767   | 8             | -1.2   | 8.6  | 0.484   |
| CD69+                    | CD56hiCD16- NK cells   | 9         | 1.2    | 8.3  | 0.282   | 8             | -3.5   | 18.7 | 0.889   |
| CD69+                    | CD56hiCD16hi NK cells  | 9         | -10.0  | 16.7 | 0.282   | 8             | -8.4   | 40.0 | 0.361   |
| CD69+                    | CD56loCD16- NK cells   | 9         | -22.6  | 41.1 | 0.110   | 8             | 0.4    | 29.1 | 0.889   |
| CD69+                    | CD56loCD16hi NK cells  | 9         | -7.4   | 18.5 | 0.086   | 8             | -8.9   | 24.6 | 0.208   |
| CD69+                    | CD56loCD16lo NK cells  | 9         | -0.2   | 21.3 | 0.767   | 8             | -6.3   | 25.8 | 0.263   |

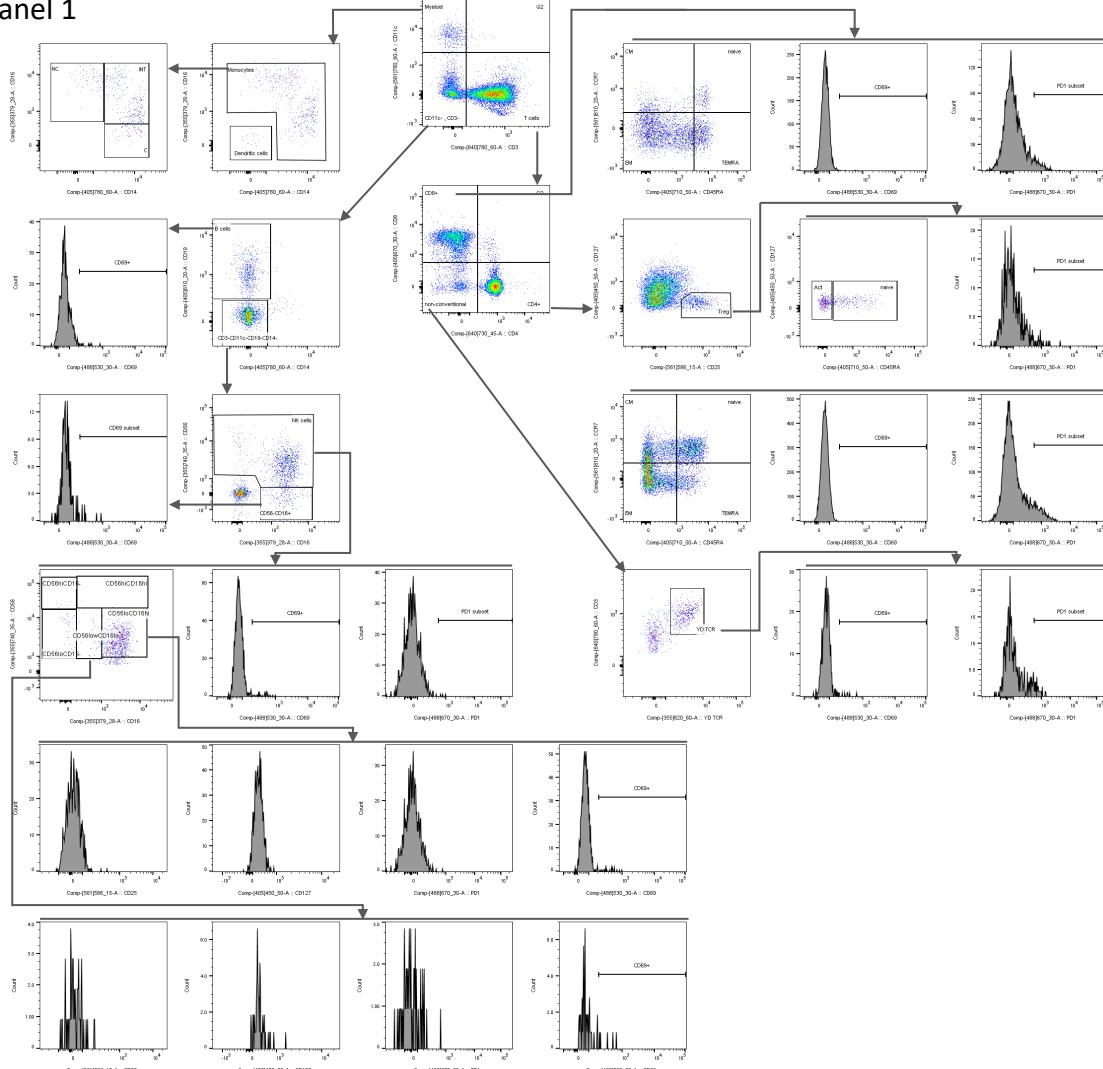
SE = stimulation effect (iSARS-iVERO)

**Supplementary table 3: Difference (3-months - baseline) in stimulation effect on immune cell marker expression levels**

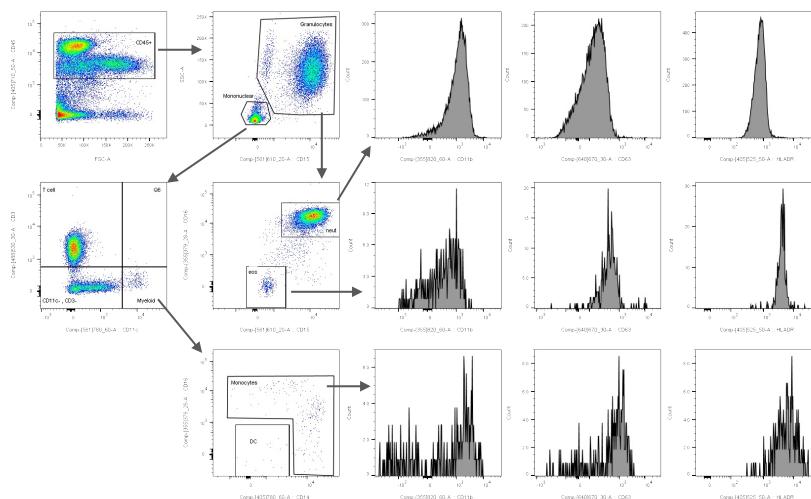
| MFI   | Parent population     | BCG group |         |         |              | Placebo group |         |         |              |
|-------|-----------------------|-----------|---------|---------|--------------|---------------|---------|---------|--------------|
|       |                       | N         | median  | IQR     | P-value      | N             | median  | IQR     | P-value      |
| CD69  | CD69+B cells          | 9         | -102.0  | 138.7   | <b>0.028</b> | 8             | -29.0   | 381.0   | 0.327        |
| CD69  | CD69+CD4+ Tconv       | 9         | 11.0    | 165.5   | 0.314        | 8             | 57.8    | 98.5    | 0.093        |
| CD69  | CD69+CD8+ T cells     | 9         | 68.0    | 134.3   | 0.374        | 8             | 84.0    | 140.0   | 0.263        |
| CD69  | CD69+NK cells         | 9         | 71.0    | 106.5   | 0.314        | 8             | -63.5   | 190.8   | 0.069        |
| CD69  | CD69+yδ T cells       | 3         | -43.5   | 723.5   | 0.285        | 4             | -359.5  | 947.5   | 0.273        |
| PD1   | PD1+CD4+ Tconv        | 9         | 16.5    | 36.0    | 0.086        | 8             | -13.5   | 40.0    | 0.093        |
| PD1   | PD1+CD8+ T cells      | 9         | 24.6    | 74.6    | 0.678        | 8             | 3.0     | 44.1    | 0.889        |
| PD1   | PD1+NK cells          | 9         | 2.2     | 35.5    | 0.441        | 8             | 15.2    | 29.6    | 0.093        |
| CD11b | Eosinophils           | 9         | 523.0   | 745.0   | <b>0.008</b> | 8             | 15.1    | 957.6   | 0.889        |
| HLADR | Eosinophils           | 9         | 163.0   | 3008.0  | 0.866        | 7             | 77.0    | 2339.0  | 0.735        |
| CD63  | Eosinophils           | 9         | 898.0   | 1010.0  | <b>0.008</b> | 8             | 673.0   | 742.5   | 0.093        |
| CD11b | Neutrophils           | 9         | 1602.0  | 476.0   | <b>0.008</b> | 8             | 1227.5  | 1656.0  | <b>0.017</b> |
| HLADR | Neutrophils           | 9         | -81.0   | 259.0   | 0.933        | 7             | 16.0    | 306.0   | 0.866        |
| CD63  | Neutrophils           | 9         | 419.0   | 191.0   | <b>0.008</b> | 8             | 307.0   | 713.5   | <b>0.012</b> |
| CD11b | Monocytes             | 9         | -1036.7 | 1525.0  | 0.086        | 8             | -1102.8 | 1276.8  | <b>0.025</b> |
| HLADR | Monocytes             | 9         | -8722.0 | 24830.0 | 0.237        | 7             | -9936.0 | 17353.0 | <b>0.018</b> |
| CD63  | Monocytes             | 9         | -1800.0 | 4758.0  | <b>0.038</b> | 8             | -2822.5 | 3136.5  | <b>0.036</b> |
| CD127 | CD56loCD16hi NK cells | 9         | 65.0    | 147.0   | 0.086        | 8             | 25.0    | 83.5    | 0.327        |
| CD127 | CD56loCD16lo NK cells | 9         | -11.0   | 216.0   | 0.859        | 8             | 44.5    | 318.8   | 0.484        |
| PD1   | CD56loCD16hi NK cells | 9         | 14.1    | 33.8    | 0.314        | 8             | 6.9     | 13.4    | <b>0.017</b> |
| PD1   | CD56loCD16lo NK cells | 9         | 16.3    | 63.5    | 0.859        | 8             | 55.3    | 59.3    | 0.161        |
| CD25  | CD56loCD16hi NK cells | 9         | 6.2     | 15.4    | 0.214        | 8             | -0.9    | 4.5     | 0.401        |
| CD25  | CD56loCD16lo NK cells | 9         | 8.5     | 29.6    | 0.594        | 8             | -5.5    | 31.2    | 0.484        |

SE = stimulation effect (iSARS-iVERO)

## Panel 1



## Panel 2



## **Supplementary figure 2: Single cell immunophenotyping manual gating strategies**